February 2019

Quick-Start Protocol

exoRNeasy Midi/Maxi Kit

Part 1: Vesicle Isolation

The exoRNeasy Midi/Maxi Kits (cat. nos. 77144, 77164, 77023) are shipped at ambient temperature. Store RNeasy® MinElute® spin columns immediately at 2–8°C. QIAzol® Lysis Reagent and all remaining components can be stored at room temperature (15–25°C).

Further information

- *exoRNeasy Midi/Maxi Handbook*: **www.qiagen.com/HB-2630**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol (Part 1 of 2) is for capture and lysis of exosomes and other extracellular vesicles (EVs). It is intended for serum or plasma volumes of 100 µl – 1 ml using the Midi kit and 1–4 ml using the Maxi kit.
- The protocol to isolate total RNA – including mRNA, miRNA and other noncoding RNAs – from EV lysates is in Part 2.
- For detailed protocols and starting volumes for other biofluids (e.g., urine, cerebrospinal fluid, cell culture supernatants), refer to the *exoRNeasy Midi/Maxi Handbook*.
- If necessary, redissolve any precipitate in Buffer RWT by warming.
- Add 30 ml ethanol (96–100%) to Buffer RWT concentrate and 44 ml ethanol (96–100%) to Buffer RPE concentrate before use.
- It is recommended to only use prefiltered biofluids, excluding particles larger than 0.8 µm (e.g., using Sartorius® Minisart® NML [cat. no. 16592] or Millipore® Millex®-AA [cat. no. SLAA033SB] syringe filters).

1. Add 1 volume Buffer XBP to 1 volume of sample. Mix well immediately by gently inverting the tube five times.
2. Add the sample/Buffer XBP mix into the exoEasy spin column, and spin the device for 1 min at 500 x *g*. Discard the flow-through and place the column back into the same collection tube.

   Note: In case any liquid remains on the membrane, spin again for 1 min at 5000 x *g* to make sure all liquid has passed through the membrane.
3. Add 10 ml Buffer XWP (Maxi) or 3.5 ml Buffer XWP (Midi), and then spin for 5 min (Maxi) or 1 min (Midi) at 5000 x *g* to wash the column and remove residual buffer. Discard the flow-through together with the collection tube.

   Note: It is possible to reduce the steps performed at 5000 x *g* down to a minimum force of 3000 x *g* without performance loss.
4. Transfer the spin column to a fresh collection tube.
5. Add 700 µl QIAzol to the membrane. Spin for 5 min at 5000 x *g* to collect the lysate, and then transfer completely to a supplied 2 ml tube.
6. Proceed to the *exoRNeasy Midi/Maxi Kit, Part 2: RNA Isolation* quick-start protocol.

Scan QR code for handbook.

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For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.